

Selective Reduction in Cortical Bone Mineral Density in Turner Syndrome Independent of Ovarian Hormone Deficiency

VLADIMIR K. BAKALOV, LAUREN AXELROD, JEFFREY BARON, LORI HANTON, LAWRENCE M. NELSON, JAMES C. REYNOLDS, SUVIMOL HILL, JAMES TROENDLE, AND CAROLYN A. BONDY

Developmental Endocrinology Branch (V.K.B., L.A., J.B., L.H., L.M.N., C.A.B.) and Division of Epidemiology, Statistics, and Prevention Research (J.T.), National Institute of Child Health and Human Development, and Warren G. Magnuson Clinical Center Radiology Department (J.C.R., S.H.), National Institutes of Health, Bethesda, Maryland 20892

Women with Turner syndrome (TS) are at risk for osteoporosis from ovarian failure and possibly from haploinsufficiency for bone-related X-chromosome genes. To establish whether cortical or trabecular bone is predominantly affected, and to control for the ovarian failure, we studied forearm bone mineral density (BMD) in 41 women with TS ages 18–45 yr and in 35 age-matched women with karyotypically normal premature ovarian failure (POF). We measured BMD at the 1/3 distal radius (D-Rad_{1/3}; predominantly cortical bone) and at the ultradistal radius (UD-Rad; predominantly trabecular bone) by dual x-ray absorptiometry.

Women with TS had lower cortical BMD compared with POF (D-Rad_{1/3} Z-score = -1.5 ± 0.8 for TS and 0.08 ± 0.7 for

POF; $P < 0.0001$). In contrast, the primarily trabecular UD-Rad BMD was normal in TS and not significantly different from POF (Z-score = -0.62 ± 1.1 for TS and -0.34 ± 1.0 for POF; $P = 0.26$). The difference in cortical BMD remained after adjustment for height, age of puberty, lifetime estrogen exposure, and serum 25-hydroxyvitamin D ($P = 0.0013$). Cortical BMD was independent of serum IGF-I and -II, PTH, and testosterone in TS.

We conclude that there is a selective deficiency in forearm cortical bone in TS that appears independent of ovarian hormone exposure and is probably related to X-chromosome gene(s) haploinsufficiency. (*J Clin Endocrinol Metab* 88: 5717–5722, 2003)

TURNER SYNDROME (TS) is caused by partial or total monosomy X and is characterized by early ovarian failure, short stature, and multiple skeletal abnormalities. The latter two features have been attributed to haploinsufficiency for *SHOX*, an X-chromosome pseudoautosomal gene involved in skeletogenesis (1). Apparent bone mineral deficit has been associated with TS, with early observations that hands of children with TS have osteopenic radiological appearance (2). Later densitometry studies suggested that osteopenia and osteoporosis occurs in adults with TS (3, 4). Recently, it became evident that the osteopenia in TS may be more apparent than real, because of the underestimation of bone mineral density (BMD) of small persons by areal densitometric methods such as dual x-ray absorptiometry (DXA). When corrected for body or bone size, the BMD values of women with TS given appropriate estrogen replacement therapy differ little from healthy matched controls (5, 6).

However, a recent study using quantitative computed tomography to assess the radius in women with TS reported a significant reduction in cortical but not trabecular BMD (7). The authors concluded that late pubertal induction or estrogen deficiency during puberty may be the cause for the decreased cortical BMD in women with TS (7). To investigate

this question, in the present study we compared the BMD of the 1/3 distal (D-Rad_{1/3}; cortical bone) and ultradistal radius (UD-Rad; predominantly trabecular bone) in women with TS and in women with karyotypically normal premature ovarian failure (POF). By controlling for ovarian hormone deficiency in this way, we hoped to find whether there was a genetic component of the cortical bone deficit in women with TS beyond the influence of ovarian hormone deficiency.

Subjects and Methods

Study subjects

Forty-one women with TS and 35 women with POF participated in this study. They were enrolled in two ongoing NICHD intramural studies: "Turner Syndrome: phenotype and genotype" and "Ovarian function in women with spontaneous premature ovarian failure" for which recruitment was mainly through notices on the Internet. The NICHD Institutional Review Board approved both protocols, and all subjects signed informed consents. The diagnosis of TS was based on typical clinical features, including short stature and POF and karyotype analysis that showed the presence of a complete or partial X-monosomy in at least 60% of the lymphocytes. Diagnosis of POF was based on at least 4 months of noniatrogenic amenorrhea, age younger than 40 yr, at least two determinations of FSH levels > 40 mIU/ml, and a normal 46XX karyotype. Karyotypes were determined by high-resolution G-banding of 50 lymphocytes. Women aged 18–45 yr were included in the current analysis. Grounds for exclusion were presence of chronic diseases or use of medications associated with osteoporosis. All participants in the study discontinued hormone replacement therapy at least 2 wk before their evaluation.

A thorough medical history including data on hormone replacement therapy (HRT) and GH treatment was obtained by structured personal interviews and questionnaires. Both groups took similar hormone re-

Abbreviations: BMAD, Bone mineral apparent density; BMD, bone mineral density; D-Rad_{1/3}, 1/3 distal radius; DXA, dual x-ray absorptiometry; HRT, hormone replacement therapy; POF, premature ovarian failure; TS, Turner syndrome; UD-Rad, ultradistal radius.

placement therapy regimens. Approximately half of the women of each group were taking oral contraceptives that contained 20–35 μg ethinyl estradiol. Approximately 25% were taking conjugated estrogens (~ 0.625 mg daily) in combination with continuous or cyclical oral progestins. The remainder used oral or transdermal estradiol and cyclical progestins. Only two of 41 women with TS had spontaneous menarche (at ages 13 and 14). On the other hand, only two of 35 women with POF did not have spontaneous menarche, and puberty was induced at ages 17 and 18 yr, respectively. Women with POF had a median duration of the ovarian failure of 1.5 yr (range, 6 months to 18 yr). Ten women with TS had received GH therapy between the ages of 8 and 16, for a period 1–6 yr. Eight women with TS had received androgen (oxandrolone) between ages 8 and 14 for a period 6 months to 5 yr as a treatment for short stature. Both groups were predominantly Caucasian and had similar level of physical activity and low levels of tobacco and ethanol use. A dietary survey conducted in a representative group of 27 women with TS and 52 women with POF revealed that the calcium intakes of both groups were similar and in the normal range. The women with TS had total daily calcium intake of 1535 ± 737 mg (mean \pm sd) vs. 1404 ± 707 mg for women with POF ($P = 0.35$). Three quarters of the women in each study group were taking >850 mg of calcium per day. All study subjects were in good general health and euthyroid as determined by physical examination and screening lab tests.

An index of overall life-long estrogen exposure (IEE) was calculated in the following way: $\text{IEE} = (\text{years with spontaneous menses} + \text{years on oral contraceptive pills} + \text{years on hormone replacement therapy}) / (\text{current age} - 14 \text{ or age of spontaneous puberty, if earlier than 14})$.

Biochemical studies

The serum levels of the following hormones and vitamins were measured after overnight fast in the patients with TS: intact PTH by immunochemiluminometric assay performed on the Nichols Advantage analyzer (Quest Diagnostic Nichols Institute, San Juan Capistrano, CA)

[reference range was 6–40 pg/ml (0.7–4 pmol/liter)]; 25-hydroxyvitamin D by competitive immunochemiluminometric assay performed on the Nichols Advantage analyzer (Quest Diagnostic Nichols Institute) [reference range was 10–68 ng/ml (25–170 nmol/liter)]; estradiol-17 β by competitive immunochemiluminometric assay performed on an Immulite 2000 analyzer (Los Angeles, CA) [reference values for untreated postmenopausal women were <20 –30 pg/ml (<73 –110 pmol/liter) and for early follicular phase <20 –84 pg/ml (<73 –308 pmol/liter)]. IGF-I was measured by chemiluminescence assay [normal adult values were 114–492 ng/ml (15–64 mol/liter)], and IGF-II was measured by ELISA [normal adult values were 405–1085 ng/ml (54–145 nmol/liter)]. Both tests were performed by Quest Diagnostic Nichols Institute. Women with POF had determination of serum levels of 25-hydroxyvitamin D in the same way.

Measurement of BMD

All women underwent measurement of areal BMD at the left forearm using a Hologic QDR-4500A dual-energy x-ray absorptiometer (Hologic, Inc., Bedford, MA) with fan-beam technology. The measurement sites were the D-Rad_{1/3}, a region 20 mm long, centered at a distance equal to 1/3 of the forearm length measured from the distal tip of the ulna, and the UD-Rad, a region nominally 15 mm in length that starts at 10 mm and extends to 25 mm proximal to the tip of the styloid process. Ninety-nine percent of the time it excludes the radial end plate. If not, then the distal end of the region is moved slightly toward the elbow, so that the endplate is excluded (Fig. 1). T- and Z-scores of BMD were calculated from manufacturer's normative data. All scans were reviewed by experienced physicians to ensure that the areas of interest were positioned correctly.

To minimize the influence of body size on the measured areal BMD we performed a volumetric transformation of the DXA data. This transformation was based on the assumption that the measured site is a cube whose volume was proportional to the second power of the projected

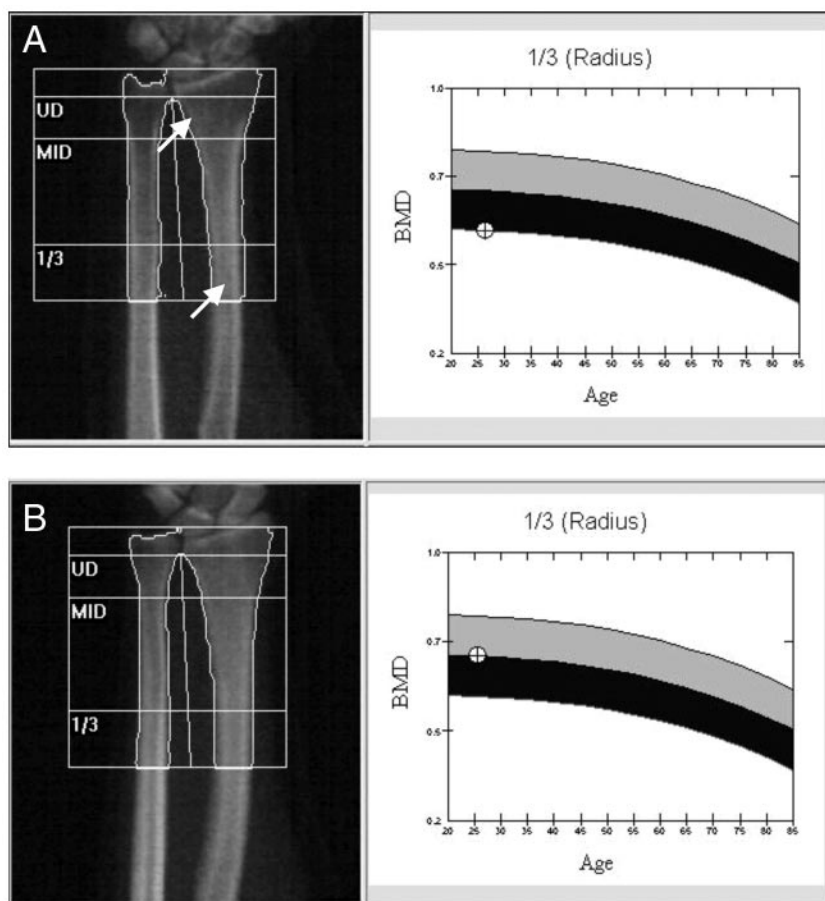


FIG. 1. DXA scans of the forearm of a woman with TS (A) and POF (B). The areas of measurement of BMD are indicated by arrows on A. UD denotes UD-Rad, a site comprising predominantly trabecular bone. 1/3 denotes D-Rad_{1/3}, a site comprising predominantly ($>95\%$) cortical bone (see *Subjects and Methods*). The right side shows the BMD of a single study subject (white dot), plotted against age-specific reference BMD (lines).

anteroposterior area of interest. The result was a volumetric density (bone mineral apparent density, BMAD), measured in grams per cubic centimeter. The following formula was applied: BMAD = bone mineral content/projected area² (8).

All women with TS had anteroposterior and lateral x-ray imaging of both wrists and distal forearms to detect Madelung's deformity.

Statistics

Data are presented as means with SDs, as medians with range, and as proportions with 95% confidence intervals, where appropriate. Comparisons between groups were performed by one-way ANOVA *t* test. Where the distribution was not normal or the variance was not equal, group medians were compared by rank-sum test. Proportions were compared by Z test with Yates correction.

The contributions of various independent variables to the variation of the BMD and BMAD were analyzed by multiple forward stepwise regression analysis and by the method of best subset regression. As a criterion for the best subset the highest adjusted R² was used. Interaction of height with BMD and BMAD was evaluated with Pearson correlation. Level of significance for all statistical procedures was accepted at *P* < 0.05. SigmaStat 2.03 (Jandel Scientific, San Rafael, CA) software was used.

Results

The majority of the women with TS had a 45, X karyotype with <30% mosaicism for 46XX or 47XXX; other karyotypes consistent with TS were similar to the distribution reported in other studies (5). All women with POF had 46, XX karyotype. The two groups were well matched in age, but women with POF were significantly taller and slightly heavier than women with TS (Table 1).

Radial BMD in TS and POF

The anatomy of the radius enables the simultaneous measurement of BMD of predominantly cortical and predominantly trabecular bone sites (Fig. 1). Measurements of BMD were done at the diaphysial distal 1/3 radius (largely cortical bone) and at an ultradistal site located 10 to 25 mm proxi-

mally from the styloid process (largely trabecular bone; Fig. 1). Thus, the close proximity of the measured sites precludes any bias from difference in gravity or muscle force.

Women with TS had a significant reduction of forearm cortical bone (D-Rad_{1/3}; Table 1) compared with age-matched women with karyotypically normal POF. The T-scores (Table 1) and the Z-scores (Fig. 2) were significantly lower in women with TS, and five women with TS qualified for a diagnosis of osteoporosis (12%; 95% confidence interval, 2%, 22%) according to World Health Organization criteria (T-score ≤ -2.5). These same parameters were within normal limits in women with POF, and none of them fulfilled the diagnostic criteria for osteoporosis (*P* = 0.04). In contrast, there was no significant difference in UD-Rad BMD in women with TS compared with POF (Fig. 2). The UD-Rad T-scores and Z-scores of both groups were close to 0, and only one woman of each group had a T-score in the osteoporotic range (T-score ≤ -2.5).

Volumetric corrections for radial BMD

There was a strong positive correlation between the BMD of D-Rad_{1/3} and the height (*r* = 0.46; *P* = 0.002) in women with TS but not in women with POF (*r* = 0.14; *P* = 0.4). To eliminate the bias of DXA toward underestimation of the areal BMD of smaller individuals, we transformed the areal BMD into volumetric BMAD (see *Subjects and Methods*). D-Rad_{1/3} BMAD was independent of height in both groups (*r* = -0.02; *P* = 0.9 for TS, and *r* = -0.15; *P* = 0.36 for POF). However, the difference in the cortical bone density (D-Rad_{1/3} BMAD) remained significantly lower in women with TS (Table 1). There was no correlation between subject's height and UD-Rad BMD in either group. The volumetric correction of the UD-Rad BMD resulted in higher BMAD of women with TS than women with POF (Table 1).

TABLE 1. Selective decrease of forearm cortical BMD in women with TS

	TS (n = 41)	POF (n = 35)	<i>P</i>
Age (yr)	32 ± 8	33 ± 5	0.52
Height (cm)	145 ± 8	164 ± 8	<0.001
Weight (kg) ^a	55 (36–101)	63 (48–93)	0.01
Body mass index (kg/m ²) ^a	26 (18–47)	22.5 (19–30)	0.002
Body surface area (m ²)	1.5 ± 0.2	1.7 ± 0.1	<0.0001
Age of menarche (yr)	15 ± 3	13 ± 2	<0.001
Index of estrogen exposure ^a	0.86 (0.07–1.0)	0.95 (0.85–0.97)	0.004
Serum 25-hydroxyvitamin D (ng/dl) ^a	24 (7–54)	23 (7–52)	0.74
D-Rad _{1/3} width (cm)	1.22 ± 0.11	1.26 ± 0.112	0.21
UD-Rad width (cm)	2 ± 0.3	2.4 ± 0.3	<0.001
D-Rad _{1/3} /UD-Rad width ratio ^a	0.61 (0.49–0.82)	0.54 (0.45–0.69)	<0.001
D-Rad _{1/3} BMD (g/cm ²)	0.59 ± 0.05	0.69 ± 0.04	<0.001
D-Rad _{1/3} T-score	-1.75 ± 0.75	-0.12 ± 0.68	<0.001
D-Rad _{1/3} Z-score	-1.5 ± 0.79	0.08 ± 0.69	<0.001
D-Rad _{1/3} BMAD (g/cm ³)	0.24 ± 0.03	0.28 ± 0.03	<0.001
Osteoporosis (T-score ≤ -2.5) n (%)	5 (12%)	0 (0%)	0.04 ^b
UD-Rad BMD (g/cm ²)	0.40 ± 0.07	0.42 ± 0.06	0.43
UD-Rad T-score	-0.73 ± 1.1	-0.42 ± 1.0	0.22
UD-Rad Z-score	-0.62 ± 1.1	-0.34 ± 1.0	0.26
UD-Rad BMAD (g/cm ³)	0.14 ± 0.04	0.12 ± 0.02	0.01
Osteoporosis (T-score ≤ -2.5) n (%)	1 (2.4%)	1 (2.9%)	0.45 ^b

To convert values of 25-hydroxyvitamin D in nmol/liter, multiply by 2.496.

^a Data are presented as median and range. Medians were compared by rank-sum test.

^b Z-test for comparison of proportions.

Factors influencing the difference in radial BMD between groups

Multiple forward stepwise regression analysis and best-subset regression analysis showed that the forearm cortical BMD (D-Rad_{1/3}) could be predicted by a linear combination of the following two independent variables: diagnosis (TS or POF), which explained 55% of the variation ($P < 0.0001$), and height, which explained an additional 5% of the variation ($P = 0.003$). The following independent variables did not contribute significantly to the variation in the D-Rad_{1/3} BMD: age, age of menarche, index of exposure to estrogens, and serum 25-hydroxyvitamin D. When the same regression model was used for BMAD instead of BMD, then only the diagnosis (TS *vs.* POF) influenced significantly the D-Rad_{1/3} BMAD.

We found a structural difference in the forearm between the two groups as well. The width of the UD-Rad was significantly reduced in TS, whereas the width of the D-Rad_{1/3} was not different in the two groups. This attenuation of

UD-Rad width resulted in significantly higher D-Rad_{1/3}/UD-Rad width ratio in women with TS (Table 1).

Factors influencing cortical BMD in TS

To further investigate the cause(s) of the selective reduction in cortical bone in women with TS, we explored the contributions of different candidate factors to the variation of D-Rad_{1/3} in women with TS. These included age, height, weight, age of starting hormone replacement, total years on HRT, index of exposure to estrogens, width of D-Rad_{1/3}, width of UD-Rad, presence of Madelung's deformity, history of GH treatment, history of androgen treatment, and current serum level of IGF-I and -II, free testosterone, estradiol, 25-hydroxyvitamin D, and intact PTH. Multiple forward stepwise regression analysis indicated that forearm cortical BMD could be explained by a linear combination of only two variables: height and history of GH treatment, each of them explaining approximately 20% of the variation. There was no difference in the D-Rad_{1/3} BMD between women who had spontaneous puberty (Z-score -1.65 ± 0.07 ; $n = 2$), and those who did not have spontaneous puberty (Z-score -1.52 ± 0.8 ; $n = 39$); $P = 0.82$. At the same time, the women with spontaneous puberty had significantly higher bone density at the UD-Rad (Z-score 1.35 ± 0.8 *vs.* -0.7 ± 1.0 ; $P = 0.006$). Surprisingly, the GH-treated patients had significantly lower cortical BMD than the nontreated subjects ($P = 0.028$; ANOVA with *t* test for difference between groups; Table 2). This difference remained even after adjustment for age, years on HRT, and timing of puberty ($P = 0.035$).

Discussion

We have shown that women with TS demonstrate a selective reduction in forearm cortical bone mineral that is not explained by ovarian hormone deficiency. This was clear from comparing cortical BMD in women with TS to women with karyotypically normal ovarian failure. If endogenous ovarian hormones are essential for normal cortical bone mineralization, then both groups of women should have a deficit, but the women with POF did not. Both groups were taking similar HRT regimens, although the TS group experienced a

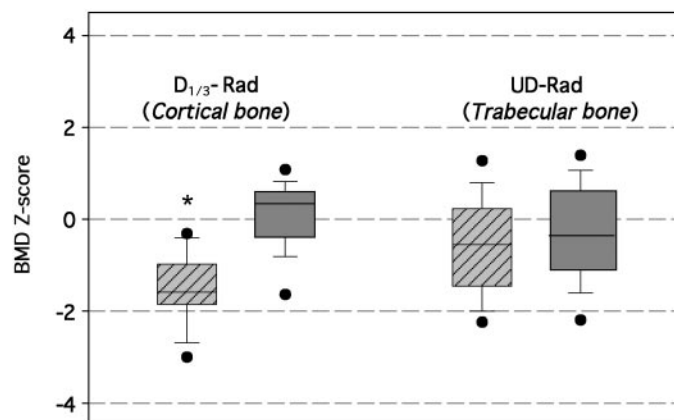


FIG. 2. Comparison of Z-scores of two forearm sites (D-Rad_{1/3}, predominantly cortical bone, and UD-Rad, predominantly trabecular bone) between women with TS (hatched boxes) and women with POF (dark gray boxes). Boxes represent the 25th and 75th percentiles. The bars represent the 10th and 90th percentiles. The black dots are the 5th and 95th percentiles. The horizontal lines represent the medians. *, $P < 0.0001$.

TABLE 2. Treatment with GH and forearm BMD of women with TS

	Treated (n = 10)	Not treated (n = 31)	P
Age (yr)	26 ± 6	34 ± 7	<0.001
Height (cm) ^a	147 (140–154)	144 (127–159)	0.8
Weight (kg) ^a	52 (39–76)	55 (36–101)	0.4
HRT starting age (yr)	16 (11–18)	16 (9–26)	0.7
Years on HRT	9 ± 7	16 ± 8	0.016
Index of Estrogen Exposure ^a	0.82 (0.25–1.0)	0.86 (0.07–1.0)	0.57
D-Rad _{1/3} width (cm)	1.24 ± 0.14	1.21 ± 0.10	0.59
UD-Rad width (cm)	1.9 ± 0.2	2.0 ± 0.3	0.18
D-Rad _{1/3} /UD-Rad width ratio	0.66 ± 0.11	0.61 ± 0.08	0.11
D-Rad _{1/3} BMD (g/cm ²)	0.56 ± 0.05	0.60 ± 0.04	0.028
D-Rad _{1/3} Z-score	-2.1 ± 0.9	-1.3 ± 0.7	0.007
D-Rad _{1/3} BMAD (g/cm ³)	0.23 ± 0.03	0.25 ± 0.02	0.058
UD-Rad aBMD (g/cm ²)	0.38 ± 0.07	0.41 ± 0.06	0.28
UD-Rad Z-score	-0.97 ± 1.2	-0.50 ± 1.0	0.24
UD-Rad BMAD (g/cm ³)	0.14 ± 0.04	0.14 ± 0.04	0.9

Medians are compared by rank-sum test.

^a Data are presented as median and range.

later (predominantly medically induced) puberty compared with the women with POF. However, the two women with TS who did have a spontaneous puberty did not have normal cortical bone density, and neither the age of puberty nor the duration of estrogen exposure contributed to variation in cortical BMD. Furthermore, there is no attenuation of BMD in the estrogen-sensitive trabecular bone in these same women. Finally, early work using plain radiographs suggested reduced phalangeal cortical bone attributed to deficient periosteal growth before the normal time of puberty (2) in children with TS. The present results obtained from analysis of cortical and trabecular bone in the forearm confirm our previous findings of normal BMD of lumbar spine vertebra (mainly trabecular bone) but significantly reduced BMD at the femoral neck (~50% cortical bone) in women with TS (6).

Bechtold *et al.* (7), using quantitative computed tomography, previously reported a reduction in cortical BMD of the radius in a study of young women with TS ($n = 20$; mean age = 19 yr) compared with normal women. Noting that the one study subject that had normal ovarian function also had normal BMD, and previous work showing an increase in cortical bone in normal girls during puberty (9), they concluded that the reduced cortical bone in TS was related to ovarian insufficiency. Based on that study and other work suggesting that androgen supports periosteal bone formation (10), we thought it possible that pubertal ovarian androgen production may have a role in cortical bone formation and that treatment of ovarian failure with estrogen alone would therefore not be sufficient for fully normal bone formation. We did not, however, find any correlation between serum testosterone levels and cortical BMD in women with TS in this study, and the women treated with androgen during peripubertal years did not demonstrate enhanced cortical BMD. Moreover, we found that women with TS that had a normal spontaneous puberty did not show any better cortical BMD than the women without spontaneous puberty who had estrogen treatment, although they did have improved trabecular BMD. Thus, data from the present study do not support a role for ovarian hormone deficiency in the selective reduction in cortical bone found in TS.

Gravholt *et al.* (5) reported alterations in calcitropic hormones in women with TS, and we also investigated these factors to determine whether any of them might explain the selective reduction in cortical BMD in TS. We did not confirm their finding of reduced vitamin D levels in women with TS. This discrepancy may be explained by differences in body composition between the TS group and the control group of age-matched normal women in that study (5). We have found that ovarian failure, whether due to TS or POF, is associated with relative increases in adipose over lean tissue compared with normal women (our unpublished results), and adiposity is associated with reduced circulating vitamin D levels (11). Thus there may be differences in vitamin D levels between women with TS and normal, eugonadal controls but evidently not in comparison with other women with ovarian failure. In any case, 25-hydroxyvitamin D and PTH levels were within

normal limits and were not correlated with BMD in women with TS in this study.

An unexpected result of this study was the finding that a history of GH treatment had a negative impact on cortical BMD in women with TS. The GH effect observed in this study was not due to delayed pubertal induction, because both groups had pubertal induction at approximately 15 yr of age and because the GH effect was independent of both age of puberty and years of estrogen exposure upon regression analysis. GH treatment is reported to increase cortical BMD in patients with GH deficiency (12) and in girls with TS (13). However, in the latter studies, there have been no untreated controls, so it is not known whether the girls would have increased their cortical BMD similarly or even more without treatment. Rats treated with GH show increased bone size and areal BMD values, but volumetric mineral density measured by quantitative computed tomography is reduced (14). The group that received GH in the present study was relatively small, and the current observations must be viewed as preliminary but cautionary. As a matter of speculation, it is possible that the molecular defect responsible for reduced cortical bone formation in TS also results in adverse effects of GH.

In summary, this study shows a selective reduction in cortical bone mineral of the forearm in TS, whereas trabecular bone mineral appears normal. Several lines of evidence suggest that this finding is not explained by deficiencies of ovarian or other endocrine systems, implicating X-chromosomal genetic factors in this skeletal defect. Many defects in skeletal development observed in TS are attributed to haploinsufficiency for *SHOX* (1), hence it is possible that *SHOX* may also be involved in normal cortical bone formation. It will be important to investigate cortical BMD in subjects with Leri-Weil syndrome to pursue this question.

Acknowledgments

Received May 28, 2003. Accepted September 8, 2003.

Address all correspondence and requests for reprints to: Vladimir K. Bakalov, M.D., 10 Center Drive, Building 10/10N262, National Institutes of Health, Bethesda, Maryland 20892. E-mail: bakalov@mail.nih.gov.

References

1. Ross JL, Scott Jr C, Marttila P, Kowal K, Nass A, Papenhausen P, Abboudi J, Osterman L, Kushner H, Carter P, Ezaki M, Elder F, Wei F, Chen H, Zinn AR 2001 Phenotypes associated with *SHOX* deficiency. *J Clin Endocrinol Metab* 86:5674–5680
2. Dalla Palma L, Cavina C 1966 Turner's syndrome: radiological appearances. *Radiol Clin Biol* 35:65–79
3. Naeraa RW, Brixen K, Hansen RM, Hasling C, Mosekilde L, Andresen JH, Charles P, Nielsen J 1991 Skeletal size and bone mineral content in Turner's syndrome: relation to karyotype, estrogen treatment, physical fitness, and bone turnover. *Calcif Tissue Int* 49:77–83
4. Davies MC, Gulekli B, Jacobs HS 1995 Osteoporosis in Turner's syndrome and other forms of primary amenorrhoea. *Clin Endocrinol (Oxf)* 43:741–746
5. Gravholt CH, Lauridsen AL, Brixen K, Mosekilde L, Heickendorff L, Christiansen JS 2002 Marked disproportionality in bone size and mineral, and distinct abnormalities in bone markers and calcitropic hormones in adult Turner syndrome: a cross-sectional study. *J Clin Endocrinol Metab* 87:2798–2808
6. Bakalov VB, Chen ML, Baron J, Hanton LB, Reynolds JC, Stratakis CA, Axelrod LE, Bondy CA 2003 Bone mineral density and fractures in Turner syndrome. *Am J Med* 115:259–264
7. Bechtold S, Rauch F, Noelle V, Donhauser S, Neu CM, Schoenau E, Schwarz HP 2001 Musculoskeletal analyses of the forearm in young women with Turner

- syndrome: a study using peripheral quantitative computed tomography. *J Clin Endocrinol Metab* 86:5819–5823
8. **Carter DR, Bouxsein ML, Marcus R** 1992 New approaches for interpreting projected bone densitometry data. *J Bone Miner Res* 7:137–145
 9. **Schoenau E, Neu CM, Rauch F, Manz F** 2002 Gender-specific pubertal changes in volumetric cortical bone mineral density at the proximal radius. *Bone* 31:110–113
 10. **Vanderschueren D, Vandenput L** 2000 Androgens and osteoporosis. *Andrologia* 32:125–130
 11. **Arunabh S, Pollack S, Yeh J, Aloia JF** 2003 Body fat content and 25-hydroxyvitamin D levels in healthy women. *J Clin Endocrinol Metab* 88:157–161
 12. **Amato G, Izzo G, La Montagna G, Bellastella A** 1996 Low dose recombinant human growth hormone normalizes bone metabolism and cortical bone density and improves trabecular bone density in growth hormone deficient adults without causing adverse effects. *Clin Endocrinol (Oxf)* 45:27–32
 13. **Sas TC, de Muinck Keizer-Schrama SM, Stijnen T, van Teunenbroek A, van Leeuwen WJ, Asarfi A, van Rijn RR, Drop SL** 2001 Bone mineral density assessed by phalangeal radiographic absorptiometry before and during long-term growth hormone treatment in girls with Turner's syndrome participating in a randomized dose-response study. *Pediatr Res* 50:417–422
 14. **Rosen HN, Chen V, Cittadini A, Greenspan SL, Douglas PS, Moses AC, Beamer WG** 1995 Treatment with growth hormone and IGF-I in growing rats increases bone mineral content but not bone mineral density. *J Bone Miner Res* 10:1352–1358